Adaptive Optics Fundus Camera to Examine Localized Changes in the Photoreceptor Layer of the Fovea

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Purpose: To examine highly localized photoreceptor disruptions in the fovea by a high-resolution adaptive optics (AO) fundus camera combined with Fourier-domain optical coherence tomography (FD OCT).

Design: Observational case series.

Participants: Three eyes of 3 patients who showed dark foveal spots by slit-lamp biomicroscopy.

Methods: Three patients who reported metamorphopsia but showed no changes in the retina in conventional fundus photographs were examined. High-resolution retinal images were obtained with the AO fundus camera and by FD OCT. The images were compared with the findings obtained by standard clinical tests, including Amsler charts and fluorescein angiography (FA).

Main Outcome Measures: Quantitative measurements of the area of photoreceptor disruption.

Results: Slit-lamp biomicroscopy revealed an irregularly shaped dark spot in the fovea centralis but no changes in FA in the 3 cases. The photoreceptor mosaic was absent in a highly localized area of the fovea in the images obtained by the AO fundus camera, and the photoreceptor outer segment was absent or disturbed at the corresponding area by FD OCT in all 3 cases. The horizontal and vertical sizes of the area of disturbance of the photoreceptor mosaic in the AO images in the 3 eyes were 400×200 μm, 300×120 μm, and 300×200 μm. These sizes were comparable to the photoreceptor outer segment disturbances in the OCT images which were 330×150 μm, 280×100 μm, 200×150 μm, respectively.

Conclusions: Localized OS disturbances were able to be detected in eyes with a dark foveal spot by AO fundus camera 2-dimensionally and by FD OCT axially. The good correspondence of the sizes of the area of photoreceptor disturbances obtained by AO images to those by FD OCT images indicate that the AO images can be used to evaluate and follow the 2-dimensional area of focal changes of the photoreceptors in the fovea quantitatively.

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With current advanced retinal imaging instruments, small focal changes of the retina can be detected and measured more accurately. These findings can help in determining the cause of unexplained visual symptoms and visual loss. For example, the retina of patients at the early phase of macular dystrophy appears ophthalmoscopically normal.[1–6] However, examination of optical coherence tomography (OCT) images showed that the retina was thinner at the macular area and that the decrease in thickness was correlated with the reduced visual acuity.[4] With additional improvements in the axial resolution by ultra–high-resolution OCT, several studies have shown a good correlation between the disruption of the photoreceptor inner segment/outer segment (IS/OS) junction and the decrease in visual acuity.[7–10] Ultra–high-resolution OCT, or Fourier-domain (FD) OCT, has an axial resolution of approximately 3 to 5 μm,[9–12] which is significantly better than the axial resolution of approximately 10 μm with the standard OCT. This increased resolution results in better delineation of the retinal architecture and helps in identifying pathologic changes in the

microstructure of the retina, especially the photoreceptor layer.[9–11]

A disturbance of IS/OS junction has been reported in cases of postoperative retinal detachment, central serous chorioretinopathy, and retinal dystrophy.[7,8,10,11,13,14] Some studies have found a good correlation between the disturbance of IS/OS junction and the visual acuity.[4,7,10,11]

One problem with conventional OCT is its low transverse resolution. Generally, the transverse resolution of OCT is on the order of 20 μm, which exceeds the cone mosaic spacing of 5 to 10 μm. Two reasons for this limitation are the ocular aberrations and saccadic eye movements. The A-scan technologies adopted in OCT are not suited for obtaining transverse information in both the x- and y-directions in a short acquisition time, and obtaining motion-artifact–free 2-dimesional transverse images of the cone mosaic is not possible.[16]

Adaptive optic (AO) systems seem to be well suited to overcome these problems. An AO system consists of a wavefront sensor to measure ocular aberrations and a de-

formable mirror to compensate for these aberrations. Correcting the ocular aberrations with the AO system can improve the transverse resolution to less than 2 μm, which is necessary to image individual photoreceptors in the living retina.[16–20] Because transverse 2-dimensional images of the retina can be obtained with the AO system, precise detection and measurements of small lesions can be made. Thus, the AO images of patients with cone dystrophy have been reported to have a patchy configuration because of photoreceptor dropout.[21–23] The limitation of the AO system is its low axial resolution. The axial resolution of AO system is approximately 100 μm, even when it is coupled with a scanning laser ophthalmoscope.[19] It is even greater with conventional flood-illumination fundus photography.

Because of the complementary aspects of FD OCT and AO, that is, high axial resolution with FD OCT and high transverse resolution with the AO system, it theoretically would be valuable to combine both instruments to evaluate small focal photoreceptor disruptions. However, the authors have not found a publication that used both systems to compare the images obtained with FD OCT and those obtained with the AO system. The authors have developed a compact, clinically friendly AO fundus camera using a liquid crystal phase modulator. With this instrument, they have been able to show the increased cone spacing in myopic eyes.[24] The purpose of this study was to determine the cause of dark spots in the fovea of 3 patients with metamorphopsia. In all 3 patients, the fundus appeared ophthalmoscopically normal and the photographs obtained by conventional fundus photography also demonstrated normal results. These retinas were examined with their custom-built AO fundus camera, and the images were compared with the OCT images.

Patients and Methods

Patients

Three consecutive patients who reported metamorphopsia but whose photographs of the ocular fundus by standard fundus photography demonstrated normal results were studied. All patients had visited Osaka University Hospital between January and June 2006. The research protocol was approved by the Institutional Review Board of the Osaka University Medical School, and the procedures conformed to the tenets of the Declaration of Helsinki. After the nature and possible consequences of the study were explained, written informed consent was obtained from all patients.

Procedures

All patients underwent a comprehensive ophthalmologic examination, including the measurement of best-corrected visual acuity (BCVA), Amsler chart, fundus photography, and slit-lamp biomicroscopy of the fundus. They also underwent examinations by FD OCT (RTVue-100; Optovue, Inc., Fremont, CA) and a custom-built AO fundus camera.[24] All 3 patients also underwent fluorescein angiography (FA) and indocyanine green angiography (ICGA).

Adaptive Optic Fundus Camera

A detailed description of the custom-built AO fundus camera has been published.[24] The principle of this flood illumination AO fundus camera was similar to that reported by Roorda and Williams.[17] Briefly, the main components of the camera were a nematic liquid crystal phase modulator (X8267–12; Hamamatsu Photonics, Hamamatsu, Japan), a Hartmann-Shack wavefront sensor (28×28 lenslets; specially made by Topcon, Co., Tokyo, Japan), and a scientific charge-coupled device digital camera (C9100–02; Hamamatsu Photonics).

The wavefront sensor measured the ocular wavefront up to the eighth Zernike order, and the phase modulator compensated for the measured wavefront aberrations. The system also is equipped with coaxial, 8-degree–wide viewing optics to identify the location and orientation of the highly magnified retinal images being observed.

Topical tropicamide (0.5%) and phenylephrine (0.5%) were used to dilate the pupil and to paralyze the ciliary muscle. The retina was illuminated with a 2-ms flash (635-nm wavelength) from a xenon arc lamp, and a retinal image was obtained with a 6-mm–diameter exit pupil. The patient was instructed to fixate a designated location on a target. Frame averaging was performed using custom software (Topcon) to improve the quality of the image. Overlapping images were merged using Photoshop (Adobe Systems, Inc., San Jose, CA). To identify the fovea, a montage of the AO images was made and superimposed on the fundus photographs and the fundus projection of OCT images.

Case Reports

Patient 1. A 39-year-old man reported metamorphopsia in his left eye which began 3 months earlier. His BCVA was 20/15 in both eyes. Ophthalmoscopy showed that the ocular fundus appeared normal in both eyes (Fig 1A). Slit-lamp biomicroscopy showed an irregularly shaped dark spot in the fovea centralis of the left eye. Amsler chart examination showed a localized area of metamorphopsia just below the fixation point.

Fourier-domain OCT demonstrated a disturbance of the IS/OS junction and OS layer (between second and third line of FD OCT) of approximately 330 μm on the horizontal scan and 150 μm on the vertical scan. The external limiting membrane layer was intact (Fig 1B,C).

The AO image showed a dark area, that is, an absence of the cone mosaic, at the fovea just above the fixation point. The shape of dark area was geographic, and the size was approximately 350 μm horizontally and 160 μm vertically (Fig 1D–F).

Patient 2. A 39-year-old man reported blurred vision and metamorphopsia in his right eye of 2 years' duration. His BCVA was 20/60 in the right eye and 20/20 in the left eye. He had been diagnosed with keratoconus in his right eye, but his vision did not improve after wearing a hard contact lens.

The ocular fundus appeared normal in fundus photographs (Fig 2A). Slit-lamp biomicroscopy showed an abnormal reflex in the macula and an irregularly shaped dark spot in the fovea centralis of the right eye. Amsler chart examination showed a central scotoma. Fluorescein angiography and ICGA did not show any abnormal findings (Fig 2B).

Fourier-domain OCT demonstrated a defect in the OS layer in the fovea that was located just under the IS/OS layer. The size of the defect was 280 μm on the horizontal scan and 100 μm on the vertical scan. The IS/OS junction was preserved but the intensity was slightly lower. The external limiting membrane and the RPE layers appeared to be normal (Fig 2C,D).

The AO image indicated a disappearance of the cone mosaic at the fovea. The dark area was oval, and the size was 300 μm horizontally and 120 μm vertically (Fig 2E–G).

Patient 3. A 62-year-old man reported metamorphopsia in his left eye of 6 months' duration. His BCVA was 20/200 in the right eye and 20/22 in the left eye. No abnormality was found in the ocular fundus in the conventional fundus photographs (Fig 3A). Slit-lamp biomicroscopy showed an irregularly shaped dark spot in the fovea centralis of the left eye. Amsler chart examination indicated a localized area of metamorphopsia just below the fixation point. The FA and ICGA results were normal (Fig 3B).

Fourier-domain OCT demonstrated an elevation of the external limiting membrane. The photoreceptor OS and IS/OS junction were not detected over an area of 200 μm on the horizontal scan and 150 μm on the vertical scan (Fig 3C,D).

The AO image demonstrated the disappearance of the cone mosaic at the foveal zone. At the fovea centralis, a relatively high reflective area without cone mosaic was observed. The area of the absence of foveal cones was approximately 300 μm horizontally and 200 μm vertically (Fig 3E,F).

Results

All 3 patients had metamorphopsia unilaterally, and the BCVA ranged 20/200 to 20/15 in the affected eye. The Amsler chart examination showed localized metamorphopsia in 2 eyes and a central scotoma in 1 eye. The ocular fundus appeared to be normal in standard fundus photographs, but slit-lamp biomicroscopy revealed an irregularly shaped dark spot in the fovea centralis in the 3 cases. Fluorescein angiography and ICGA did not show any abnormal findings in any cases.

Fourier-domain OCT demonstrated an absence of the OS and IS/OS junction of the photoreceptors in 1 case and an absence or disturbance of the OS but preservation of the IS/OS junction in 2 cases. The AO images indicated the absence of the cone mosaic in the foveal zone in all 3 cases. The horizontal and vertical sizes of the area of the absence of the photoreceptor mosaic in the AO images in the 3 eyes (400×200 μm, 300×120 μm, and 300×200 μm) were comparable with the sizes of the photoreceptor OS disturbances in the OCT images (330×150 μm, 280×100 μm, 200×150 μm, respectively).

Discussion

This study examined the FD OCT and AO images in patients who showed localized disturbances in the photoreceptor layer. To the best of the authors' knowledge, this is the first study that compares the FD OCT and AO images in the same patient. A localized disappearance of the photoreceptor mosaic was observed in the photographs of the fovea obtained by the AO fundus camera in all 3 cases. The horizontal and vertical sizes of the area of the loss of the photoreceptor mosaic in the AO images were comparable with the area of the OS disturbance in the OCT images. In patient 3, the IS/OS junction and OS were also not detected, but in patients 1 and 2, the IS/OS line was preserved, although the intensity was slightly lower than normal. These results suggested that the dark area seen by slit-lamp biomicroscopy corresponded with an absence of the photoreceptor mosaic in the AO images and with the disturbed photoreceptor OS in the OCT images.

The origin of the high reflectance cone mosaic in the AO fundus camera is reported to be from both the IS/OS junction and the OS in the normal retina.[15] However, based on the results from patient 1, a possibility exists that the OS is more involved in the reflectance of photoreceptor mosaic than the IS/OS junction in the AO images. This hypothesis corresponds with the results of a recent report using AO OCT, in which the cone mosaic is observed clearly at the level of Verhoeff's membrane (the third blight line of FD OCT), where the tip of the cone photoreceptor OS is enveloped by microvilli.[25]

In patients with a macular hole, metamorphopsia is a frequently reported symptom. In the early stage of macular hole, morphologic changes are observed not only in the photoreceptor layer, but also in the inner retinal layers because of the tangential traction on the retinal surface. All of the patients reported metamorphopsia, but the lesion was confined to only the photoreceptor layer and was in a very restricted area in the fovea.

Recently, a new clinical entity termed *foveal spot* or *macular microhole* has been proposed.[26,27] In this lesion, the patient has a mildly reduced visual acuity, a central scotoma, and metamorphopsia, and ophthalmoscopy shows a foveal defect with a red appearance and well-defined margins. The size of the lesion is approximately 100 μm and seems to be intraretinal. Conventional OCT3 images (Stratus model 3000; Carl Zeiss Meditec, Humphrey Division, Dublin, CA) show an abnormality of the outer retina, a defect of retinal pigment epithelium, or both.[27] All 3 of the eyes had an apparently normal ocular fundus by conventional fundus photography, but slit-lamp biomicroscopy showed an irregularly shaped dark spot in the fovea centralis. Thus, the 3 eyes may be included in the category of macular microhole or foveal spot. The decrease in the visual acuity or an increase in the area of metamorphopsia should be reflected in the size of the dark area in AO fundus image, and thus may be helpful to evaluate the progression of a disease quantitatively.

The limitation of this study is that the fovea could not be resolved accurately with the AO system. The AO system allows a transverse resolution of 2 μm, but the photoreceptors in the fovea are smaller than the resolution limit.[16–20] Because of this, it is difficult to identify the individual cones in the fovea centralis in the AO images. However, because the dark area is not observed in the central fovea in normal eyes, the dark area at or around the central fovea can be assumed to be the area of photoreceptor loss or disruption.

In conclusion, the AO fundus camera can acquire 2-dimensional images of the retina with a resolution of approximately 2 μm. This resolution allows the detection of highly localized disturbances of the photoreceptor cells that can correlate with the high-resolution images obtained by FD OCT. Combining the AO fundus camera and FD OCT images can be valuable to assess photoreceptor disruptions, especially in eyes with a small focal foveal lesion. The findings in these 3 patients indicate that patients reporting metamorphopsia may have a localized disruption the of photoreceptor cells in the fovea.
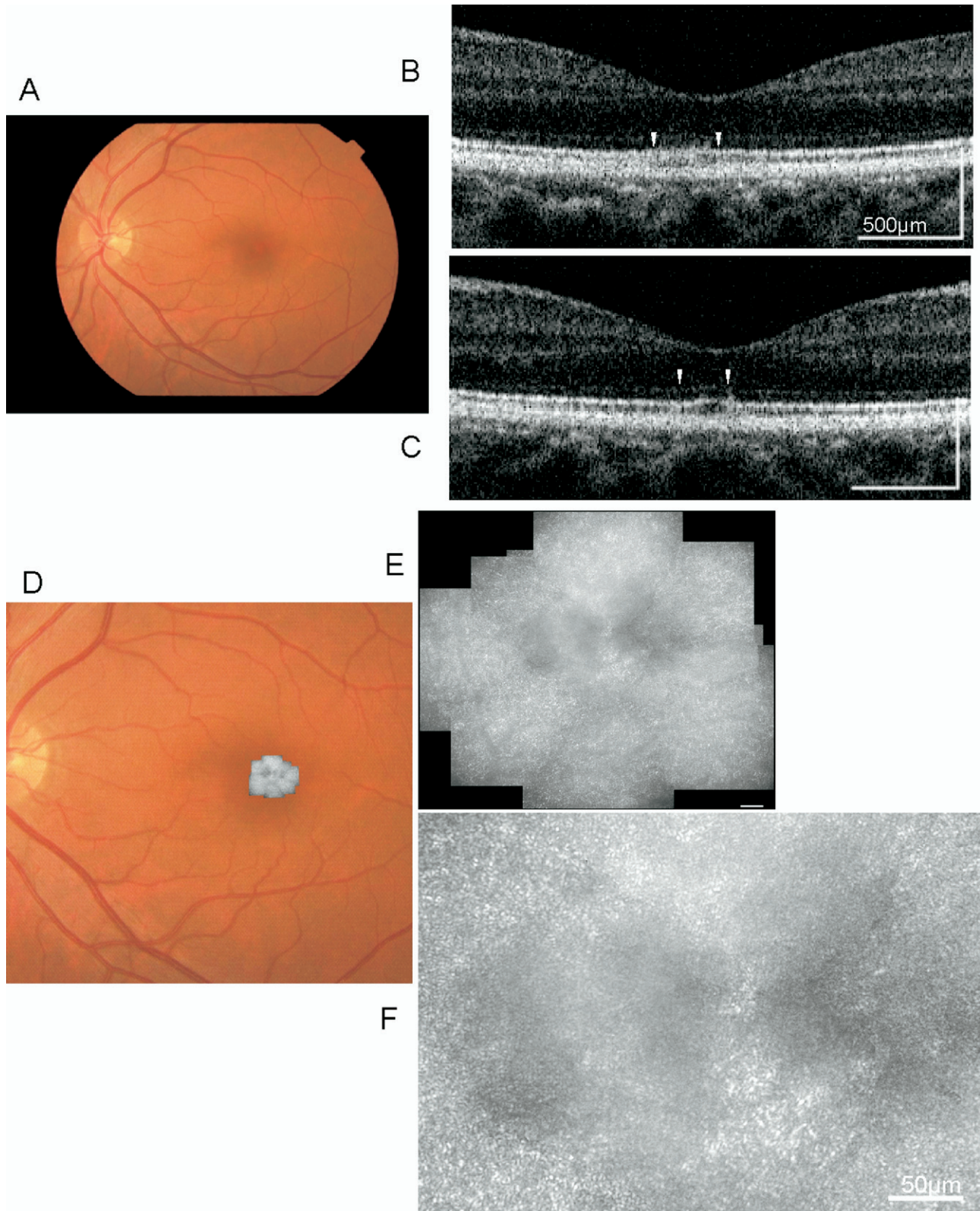
Figure 1. Images from the left eye of patient 1, who sought treatment for metamorphopsia. **A**, Fundus photograph showing normal appearance. **B**, Fourier-domain optical coherence tomography image (5-mm horizontal scan) demonstrating the disturbance of the inner segment/outer segment (IS/OS) junction and outer segment (OS) layer (between the second and third lines) for approximately 330 μm. **C**, Fourier-domain optical coherence tomography vertical scan showing the disturbance of the IS/OS junction and OS layer for approximately 150 μm. The arrowhead in **B** and **C** point to the area of IS/OS and OS disturbances. The horizontal bars in **B** and **C** represent 500 μm. **D**, Montage of adaptive optics (AO) image superimposed on the fundus photograph. **E**, Montage of adaptive optics image (low magnification). **F**, Magnified AO image of the fovea showing a dark area (disappearance of cone mosaic) at the fovea just above the fixation point. The shape of dark area was geographic and the size was approximately 350 μm horizontally and 180 μm vertically. The horizontal bars in **E** and **F** represent 50 μm.
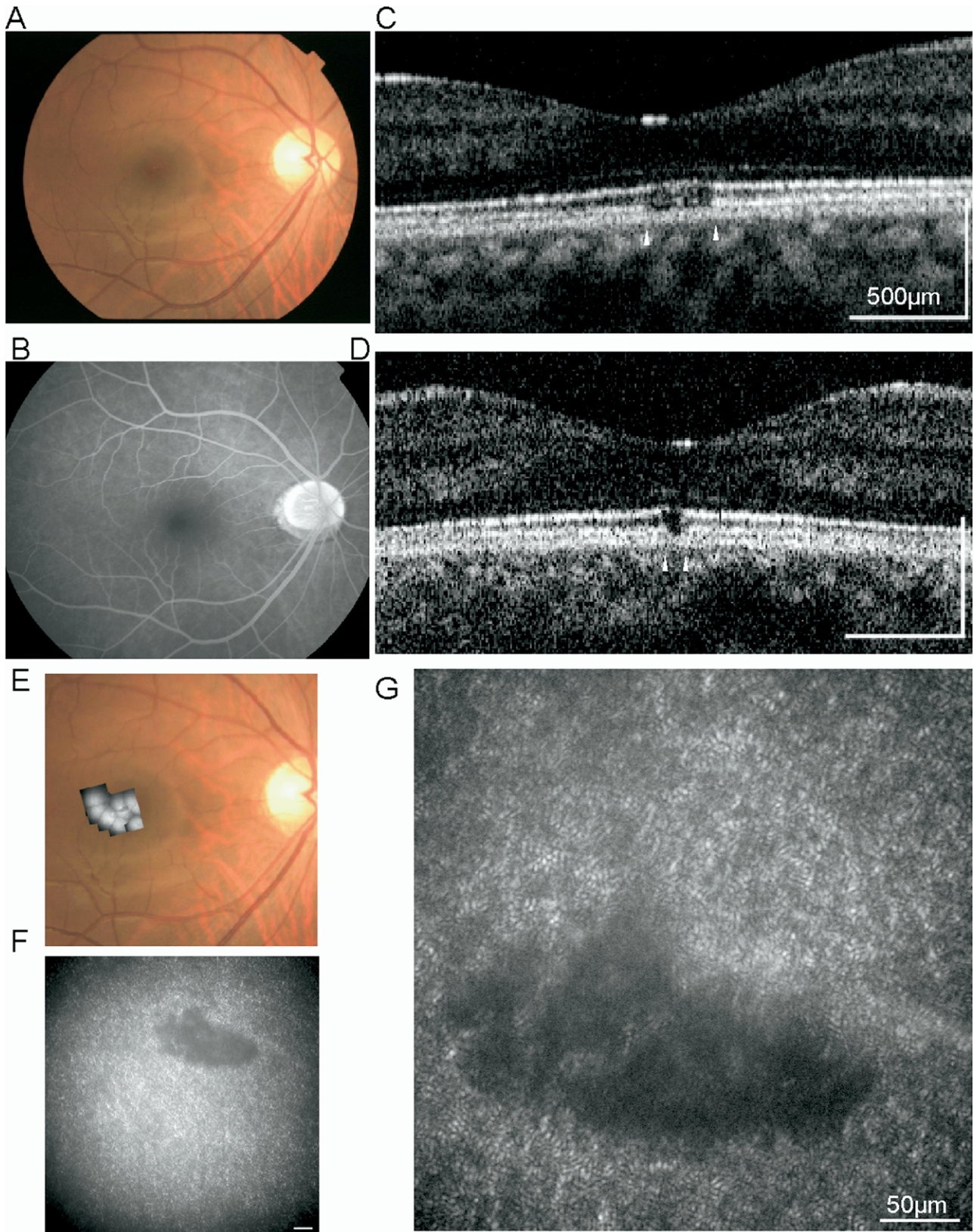
Figure 2. Images from the right eye of patient 2, who sought treatment for metamorphopsia. **A,** Fundus photograph showing that the retina appears to be normal. **B,** Early-phase fluorescein angiography image showing normal results. **C** and **D,** Fourier-domain optical coherence tomography images demonstrating a defect of outer segment (OS) layer in the fovea that was located just beneath the inner segment/OS junction. The size of the defect was (**C**) 280 μm on the horizontal scan and (**D**) 100 μm on the vertical scan. The IS/OS line was preserved but the intensity was slightly low. The arrowhead in **C** and **D** points to the area of OS defect. The horizontal bars in **C** and **D** represent 500 μm, and the vertical bars represent 200 μm. **E,** Montage of adaptive optics (AO) image superimposed on the fundus photograph. **F,** adaptive optics image (low magnification). **G,** Magnified AO image of the fovea showing a dark oval-shaped area (disappearance of cone mosaic) with a size of 300 μm horizontally and 120 μm vertically. The horizontal bars in **F** and **G** represent 50 μm.
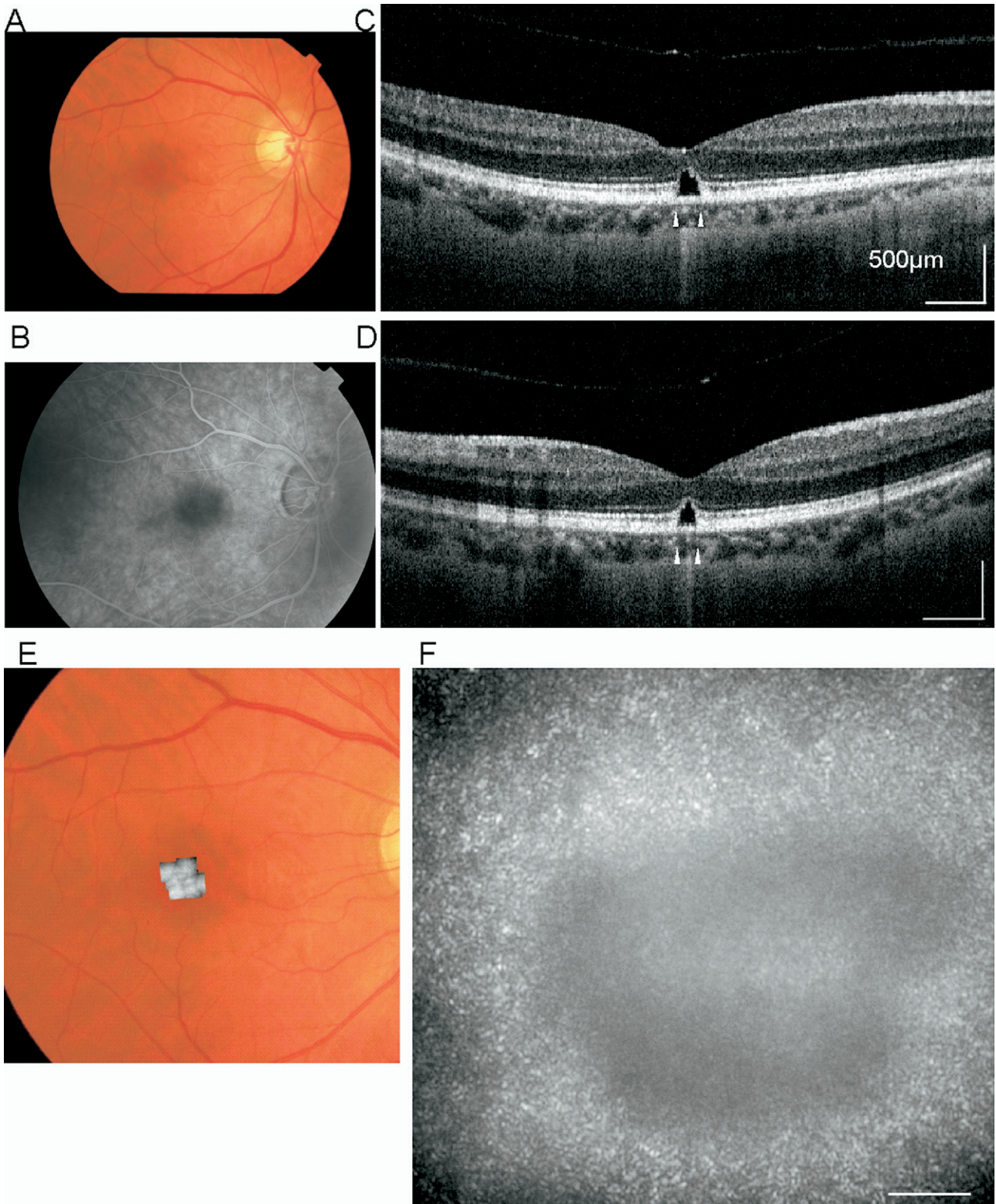
Figure 3. Images from the right eye of patient 3, who sought treatment for metamorphopsia. **A**, Fundus photograph showing normal appearance. **B**, Early-phase of fluorescein angiography image showing normal results. **C** and **D**, Fourier-domain optical coherence tomography images demonstrating the elevation of the external limiting membrane. Photoreceptor outer segment (OS) and inner segment/OS junction are not present in an area of (**C**) 200 μm on the horizontal scan and (**D**) 150 μm on the vertical scan. The arrowhead in **C** and **D** indicates the area of OS defect. The horizontal bars in **C** and **D** represent 500 μm, and the vertical bars represent 200 μm. **E**, Montage of adaptive optics (AO) image superimposed on the fundus photograph. **F**, Magnified image of AO image in the fovea demonstrating a dark oval-shaped area (disappearance of cone mosaic) with a size of 300 μm horizontally and 200 μm vertically. At the fovea centralis, a slightly high reflective area without cone mosaic was observed. The horizontal bars in **F** represent 50 μm.

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Footnotes and Financial Disclosures

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